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A DETERMINATION OF THE FREQUENCY OF ANEUPLOIDY IN CHINESE HAMSTERS (*CRICETULUS GRISEUS* MILNE-EDW.)

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A DETERMINATION OF THE FREQUENCY OF ANEUPLOIDY IN CHINESE HAMSTERS (CRICETULUS GRISEUS MILNE-EDW.)

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SUMMARY

The purpose of this study was to determine the rate of spontaneous aneuploidy in spermatogonial tissue of the Chinese hamster (Cricetulus griseus Milne-Edw.).

Aneuploidy is more reliably demonstrated by hyperdiploidy than by hypoploidy (Ford, 1964). The diploid karyotype of the Chinese hamster is composed of 22 chromosomes, four of which are very large and morphologically distinguishable. These four chromosomes were used as "marker" chromosomes in determining the state of aneuploidy. In this study, any cells having "marker" chromosomes in excess of four were scored as aneuploid and a karyotype was constructed. In addition, cells containing one more or one less than the four "marker" chromosomes were recorded in combination with the total chromosome number, and a determination was made of the frequency of gain or loss of one "marker" chromosome combined with the occurrence of chromosomes from 20 to 24. Chromosome counts and karyotypes were made from premeiotic cells prepared by a modification of the method of Conger and Fairchild (1953).

Frequency of aneuploidy of the trisomic type in the spermatogonial tissue of the Chinese hamster was found to be 7 in 2,250 cells for the four large "marker" chromosomes and, therefore, the frequency of trisomy involving any of the 22 chromosomes was estimated as 1 in 58.

CHAPTER I

INTRODUCTION

In the process of studying cells, whether *in vivo* or *in vitro*, total chromosome numbers differing from the normal somatic number often have been observed (Blakeslee, 1921 and Hayflick, 1965). These heteroploid cells may be divided into two groups: euploids, which contain an even multiple of a basic monoploid number and aneuploids, which do not. 2n refers to the diploid chromosome number while the haploid number is n.

Cells with a chromosome complement of 2n + 1, 2n + 2, 2n + 1 + 1, 2n - 1, etc. are aneuploids and are labeled trisomic, tetrasomic, double trisomic and monosomic respectively (Burnham, 1962). The general term for cells having between 2n and 3n or more chromosomes is hyperploidy; cells having chromosome numbers between n and 2n are hypoploid.

The effects of aneuploidy on the phenotypic expression of organisms was first observed around the early 1900's in plants such as *Datura* and *Oenothera* which were used for cytological studies. Blakeslee had been growing *Datura stramonium* for class demonstrations at Storrs, Connecticut, since 1910 (Blakeslee, 1922). About five years later, he noticed the first "mutant" plants that were more feeble than normal, had a high frequency of pollen sterility and a characteristic globe shape. Test crosses indicated the globe shaped characteristic was dominant and was transmitted through the female; however, this characteristic did not breed true neither did it occur in the expected frequency. It was not until 1920 when he and his co-investigators began to study the cytology

of the "mutants" that they discovered an extra chromosome, i.e., 2n + 1 (trisomic), the normal diploid number being 24 (Blakeslee, Belling and Farnham, 1920). Similar observations were made on Oenothera plants. Davis (1916) and other investigators noticed that Oenothera Franciscana demonstrated seed sterility and Oenothera biennis demonstrated pollen sterility. Cytological studies of Oenothera plants showed that the chromosomes of all species form rings during meiosis and, consequently, non-disjunction frequently occurs producing hybrid offspring or sterile pollen grains and seeds (Cleland, 1923 and Darlington, 1965). The amount of viability and phenotypic variation of the "mutants" is probably related to which chromosome of the cell complement is extra and, therefore, which genes are in excess.

Aneuploidy is a fairly unusual condition in animals according to Darlington (1965). Nevertheless, aneuploidy was observed in the oogonial cells of the fruit fly Drosophila melanogaster by Bridges in 1921. Moreover, Wilson (1925) observed an extra Y-chromosome (a super-numerary) in some spermatogonial cells of the insect Metapodius terminalis which was produced by non-disjunction. Nabours (1914) in his study of Parattetix (grasshoppers) found nine distinct true-breeding wild types with different body colors. In one of his experiments, Nabours crossed a BI hybrid female to a CE hybrid male which gave, as expected, equal numbers of the hybrids BC, BE, CI, and EI, and in addition gave an individual of the appearance BIE. Bridges (1916) believes this individual must have occurred as a result of non-disjunction, where both the B and I bearing chromosomes remained in the egg and fertilization occurred with an E sperm. Consequently, the BIE individual should have three homologous chromosomes, and a total of one more than the number characteristic of

the species. However, Bridges did not rule out linkage as another explanation. Nabours was unable to study the individual further since it escaped.

Some genetic studies have also been done on pigeons and fowl. Cole (1912) studied the manner of inheritance of the common colors of pigeons such as black, dun, red, yellow, blue and silver. His experiments strongly indicated to him that inheritance in the blond and dark types of pigeons was an example of sex-linkage. Nevertheless, Bridges (1916) believes "non-disjunction offers an alternative explanation which seems more plausible." Bateson and Punnett (1911) studied the inheritance of pigmentation in the silky fowl and found several exceptions to Mendelian genetics which Bridges (1916) believes could be attributed to non-disjunction. Finally, Ford (1964) reported the occurrence of aneuploidy involving the T6 chromosome in normal somatic tissue of CBA mice.

Aneuploidy has been studied extensively in human cell populations as well. Jacobs, Brown and Doll (1961) found evidence of increased frequency of aneuploidy in cultures of leucocytes from human peripheral blood. Further analysis indicated that the effect was primarily attributable to an increase of apparent XO hypoploid cells with increasing age of the subject, a condition implying the loss of X-chromosomes in females and Y-chromosomes in males (Jacobs et al., 1963). Trisomics in man came to the attention of physicians and cytologists because of their resulting mental and physical abnormalities. Mongolism (Down's syndrome) demonstrates trisomy of chromosome number 21 in man. This chromosome is very small and since it is attached to the nucleolus it may fail to pair at meiosis in older women. Consequently, eggs with

two such chromosomes, when fertilized by normal sperm, give trisomic 21 embryos which survive at birth (Darlington, 1965).

It is difficult to discuss the genetic significance of aneuploidy without mentioning the somatic results because they are so closely related. By studying the phenotypic expression resulting from loss or gain of one particular chromosome the genetic content of that chromosome can be determined. When aneuploidy was first discovered it was not considered a universal phenomenon of all chromosomes. Only after more and more trisomics were discovered was the significance of aneuploidy realized. The importance of aneuploidy or more basically the importance of gene balance in evolution was recognized by Blakeslee and Darlington among others. Blakeslee (1921) performed numerous experiments to determine the amount of genetic contribution gained from whole chromosomes or large segments of them. Darlington (1929) studied the structure and variation in the chromosome complement in plants of the genus Tradescantia by observation of chromosome behavior at meiosis and its result in the gametophyte stage. He determined the types of abnormal chromosome behavior such as ring formation, lateral pairing and fragmentation that explain the variation among the Tradescantia species.

The presence or absence of chromosomes in a cell complement was sufficient evidence to explain new phenotypes as seen in Datura, Tradescantia and Oenothera. However, the non-Mendelian behavior of the "mutants" was puzzling until it was realized a large proportion of the seeds with a 2n + 1 karyotype were either sterile or did not germinate as well as normal seeds (Belling and Blakeslee, 1924). A study of Datura "mutant" pollen grain viability by Blakeslee and Cartlidge (1926) showed

that "the lack of any one chromosome caused the abortion of all gametophytes affected while the presence of an extra chromosome (2n + 1) does not." The presence of extra chromosomes causes complications such as ring and triangle formation at meiosis, especially during the stage of chromosome pairing as noted by Belling and Blakeslee (1924). From studies of Datura they found three types of trisomics which could be differentiated at meiosis: the univalent type of trisomics were rod-shaped, the secondaries were usually ring-shaped and the tertiary trisomics were seen as a chain of five chromosomes. The primary rod-shaped trisomics indicate the cell has an extra whole chromosome which resulted from nondisjunction or unequal shuffling of the chromosomes during the diakinesis stage of meiosis. Belling and Blakeslee (1924) assumed the extra chromosome in the secondary trisomic was a double half chromosome, i.e., one arm had duplicated to form a full size chromosome. This assumption was necessary to explain the configuration at meiosis, either a ring of three chromosomes or a U-shaped ring formed by the univalent. They interpreted the extra chromosome in the tertiary trisomic as an interchange, i.e., a chromosome resulting from a segment of one chromosome being replaced by a segment from a non-homologous chromosome.

Mongolism in man is one of the phenotypic effects of aneuploidy and is characterized by coarse facial features and hair, almond eyes and mental deficiency. The study of the incidence of Mongolism in metropolitan areas has been the project of several investigators. Collman and Stoller (1962) reported on the incidence of Mongolism in Victoria, Australia. They found no correlation with the sex of the child but did find that the incidence of Mongolism increased with the age of the

mother. Collman and Stoller were not willing to attribute Mongolism solely to genetic factors, expressing the opinion that an infectious agent, possibly a virus, might also be responsible for the occurrence of Mongolism. Stark and Mantel (1966) analyzed Michigan birth certificates, hospital records and other sources, and Carter and MacCarthy (1951), who studied the incidence of Mongolism in ten London hospitals, also found that the incidence of Mongolism increased with the age of the mother.

Matsunaga (1966) in his study of Mongolism in Japan indicates aneuploidy as a probable cause and stated that, "Since the overwhelming majority of our cases were trisomic for chromosome #21, the results presented are largely in cases with primary non-disjunction of that chromosome." Laboratory technique may be responsible for some, but not all of the cases being karyotyped as trisomic for number 21. Moreover, chromosome number 21 may have been involved in translocations with other autosomes. Matsunaga also noted that the incidence of Mongolism increases with the age of the mother.

Several other examples of aneuploidy giving rise to sexual aberrations due to a deviation from the normal sex-chromosome complement of XX in females and XY in males also have been studied. Turner's (XO) syndrome is characterized by gonadal dysgenesis, short stature, a webbed neck and female morphology (Money, 1968 and Federman, 1967). The Klinefelter syndrome is a consequence of an XXY condition and results generally in tall slender men who are mentally deficient. The XYY condition has also been discovered in men who exhibit undisciplined behavior (Money, 1968). The triple-X syndrome produces both mentally retarded and normal females.

Hermaphroditism is one of the most studied and documented types of

abnormal sexual development due to chromosome aberrations and resulting hormone imbalance. Most true hermaphrodites have a 46/XX karyotype which is indistinguishable from a normal female's but external appearance can be either male or female. Mosaicism, where some of the body cells are XX and the majority are XY and vice versa, is common in hermaphrodites (Federman, 1967). There are many variations in karyotypes that also result in these syndromes or combinations of them. Studies with plants such as Daturas have demonstrated that any chromosome of a plant cell is capable of demonstrating aneuploidy (Belling and Blakeslee, 1924). Nevertheless, as Darlington (1956) pointed out, in the more delicate animal and especially mammalian reproductive systems the majority of possible aneuploidic variations are not exhibited because of some inherent lethality resulting in reduced viability of male or female gametes or the zygote formed.

The purpose of this study was to determine the spontaneous rate of aneuploidy in the spermatogonial tissue of the Chinese hamster. Once this frequency is known accurately it can be used as a base line during a study of the effects of radiation on the chromosome complement of somatic tissue cells. The Chinese hamster has a diploid number of twenty-two chromosomes. Four of the twenty-two chromosomes are very large in comparison to the other eighteen and, therefore, are easily distinguishable both when scanning cells on slides and when karyotyping the cells. Consequently, these four large chromosomes pairs 1 and 2 (see Figure 1), were used in this study as "marker" chromosomes.

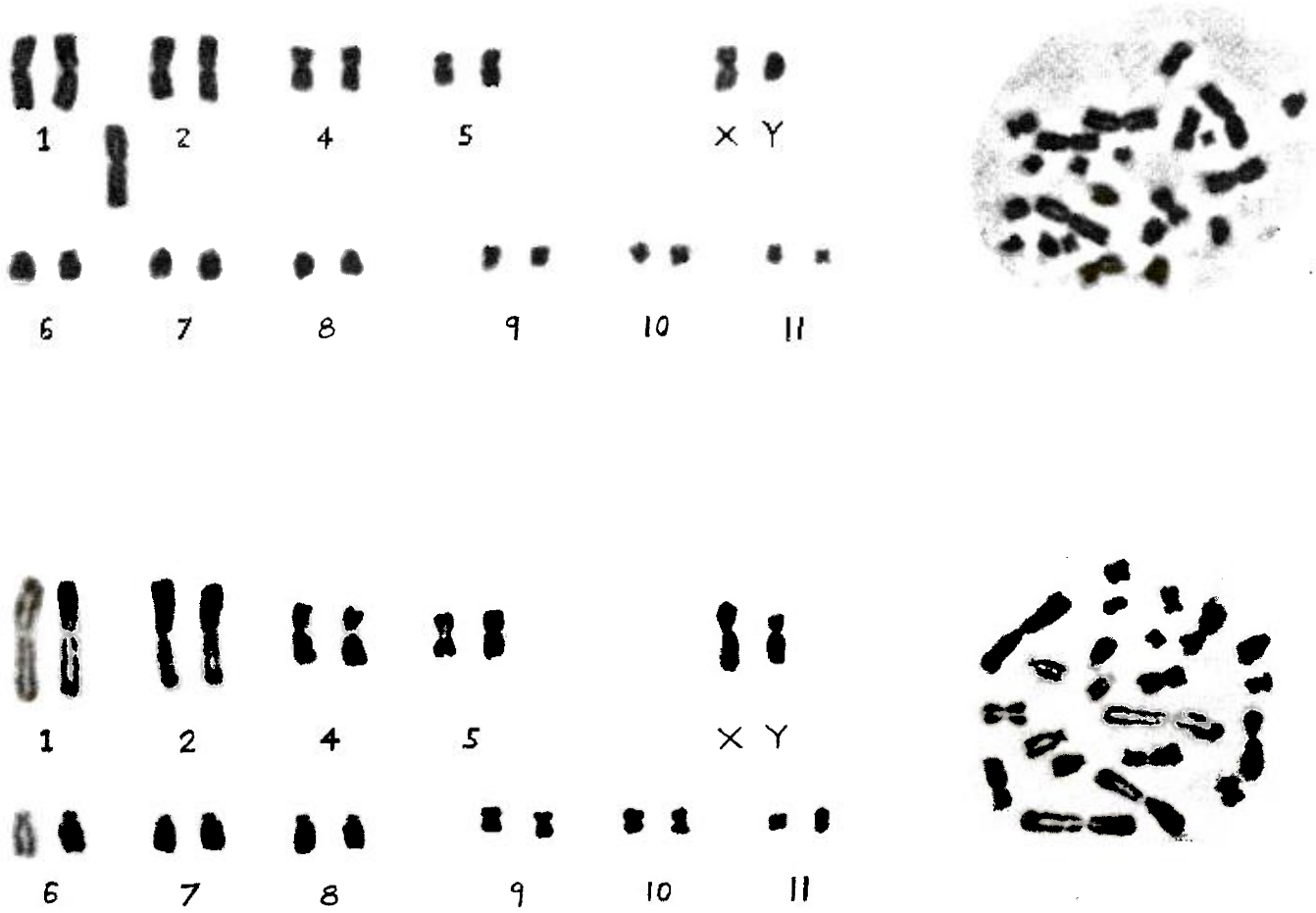

Figure 1. An Example of the Karyotype of a Trisomic and a Normal Cell from Chinese Hamster Spermatogonial Tissue.
The karyotype on top is an example of a cell trisomic for one of the large "marker" chromosomes since it has an extra chromosome (placed below and between pairs 1 and 2) which can belong to either pair 1 or 2. The lower karyotype is that of a normal cell.

CHAPTER II

MATERIALS AND METHODS

The Chinese hamsters (Cricetulus griseus Milne-Edw.) used in this study were offspring of stock from animals originally obtained from the colony of Dr. G. Yerganian of the Children's Cancer Research Foundation, Boston, Massachusetts. Only male animals three to five months of age and produced in the seventh to nineth inbred generation were used.

Spermatogonial tissue was prepared after the method of Brooks and Lengemann (1967). Three to five hours before the animals were sacrificed, they were injected intraperitoneally with 0.3 ml of 1 percent (w/v) colchicine in aqueous solution. They were killed by cervical dislocation after carbon dioxide anesthesis. The testes were then dissected and placed in (pre-warmed 37° C) physiological saline, the tunica was cut, and the seminiferous tubules teased apart. Several tubules at a time were gently pressed between a slide and a coverslip to remove spermatogonia and the tubules were washed into another solution of (pre-warmed 37° C) physiological saline. The tubules were then transferred to a hypotonic 0.7 percent (pre-warmed 37° C) aqueous sodium citrate solution for twenty minutes. Finally, the tubules were placed in a fixative of (1:1) ethyl alcohol and acetic acid and fixed overnight or longer.

For microscope slide preparations the tubules were transferred to a (3:1) solution of ethanol and acetic acid for twenty minutes. During this time the orcein dye solution was made by placing 0.2 to 0.3 grams of orcein dye in a test tube with about 10 ml of 50 percent

propionic acid and heated to boiling. After boiling, the solution was cooled and filtered. (The dye solution should be made daily.) Well flattened preparations were made by placing a few tubules at a time on a slide with a few drops of the ethanol-acetic acid solution and gently squashing the tubules with a coverslip. The slide with the coverslip was placed on dry ice for an hour or until frosted over. The coverslip was then flipped off and a few drops of the ethanol-acetic acid solution were placed on the slide and quickly burned off. When the slide cooled a few drops of the orcein dye solution were placed on the slide, a coverslip was added, the slide blotted dry and then sealed with printer's wax.

Slides were made permanent by freezing them on dry ice for an hour, flipping off the coverslip and placing them in 95 percent ethanol for one to five hours, then transferring the slides to a (1:1) solution of 95 percent ethanol and toluene for at least four hours. The slides were kept 24 hours each in two toluene solutions to clear the tissue, and mounted in Permount (Conger and Fairchild, 1953).

The counting of cells was performed using a 40X objective and phase contrast optics of a Leitz Wetzlar microscope. Cells with less than three or more than five of the large "marker" chromosomes of pairs 1 and 2 were disregarded as well as any cells that had a total chromosome count of less than twenty. These cells were considered extremely abnormal and biologically non-viable since, "On theoretical grounds the monosomic cell would be expected to have a lessor viability than the corresponding trisomic cell, if it is viable at all." (Ford, 1964). If this is true for a monosomic cell then a double or triple

monosomic also would be non-viable. Moreover, these cells could have easily suffered cell breakage and as Ford (1964) says, "... this may result in a lower count than the true one; and sometimes a single chromosome, or group of two or three, released by fracture of the cytoplasm of the parental cell and floating free, may come to rest among the chromosomes of another." Thus, one daughter cell would be lacking one to several chromosomes and the other daughter cell would have one to several chromosomes in excess.

As seen in Table 1. the total chromosome number of each cell was recorded and each was either designated normal (containing the four large "marker" chromosomes of pairs one and two), plus one (having one extra large "marker" chromosome) or minus one (having one less larger "marker" chromosome). Any cells suspected of having five large "marker" chromosomes were examined, photographed using a 100X objective and phase contrast optics of a Carl Zeiss microscope and, finally, karyotyped from the photographic prints.

Ford (1964) has demonstrated that hyperdiploidy is more reliable than hypoploidy in indicating aneuploidy. In this study both types of cells designated either as plus one or minus one were recorded. The category labeled minus one should be less significant as an indication of aneuploidy because cells can lose chromosomes easily in the squash preparation of slides. However, a cell that had an extra chromosome would almost definitely have gained it through non-disjunction to produce the aneuploidic condition. There is some chance that chromosomes could "drift" from one cell to another during the squash preparation but according to Ford (1964) "... the great uniformity of fixation

Table 1. Chromosome Counts in Normal Spermatogonial Tissue of The Chinese Hamster

Chromosome No.: / Hamster No.	Cell Count 20	21	22	23	24	Total	"Marker" Chromosome Frequency Minus One (Monosomic, 2n + 1)	Plus One (Trisomic, 2n + 1)
I	47	67	155	11	0	280	35	0
II	28	23	35	3	0	89	16	2
III	102	135	446	12	0	695	44	3
IV	64	119	296	8	0	487	21	1
V	17	19	46	3	0	85	4	1
VI	16	27	96	2	0	141	5	1
VII	73	125	268	6	0	472	29	1
VIII	46	76	243	5	0	370	18	0
Total	393	591	1585	50	0	2619	172	9

quality, depth of stain, and degree of contraction exhibited by the chromosomes of a given cell and the difference in these respects between one cell and another usually permit detection of a 'stranger' chromosome." The data seems to support this assumption since 1,585 monosomic cells were observed while only 50 trisomic cells were seen of which nine were trisomics containing the extra "marker" chromosome.

The method of determining the actual frequency of aneuploidy was based on the assumption that any chromosome can demonstrate non-disjunction during the metaphase stage of mitosis to produce two daughter cells, one of which will appear trisomic and the other monosomic at the metaphase stage of the next division. The frequency of aneuploidy is calculated by dividing the total number of cells observed by the number of trisomics observed to obtain the frequency of aneuploidy per cell. Finally, to obtain the frequency of aneuploidy involving any one of the chromosomes, the observed frequency of aneuploidy per cell must be divided by the diploid number of chromosomes and multiplied by two since either chromosome of a pair could demonstrate non-disjunction to produce the trisomic condition (Ford, 1964).

Figure 1 shows the karyotypes of a trisomic and a normal cell. The method of karyotyping and the system of chromosome pair numbering was obtained from Hsu and Benirschke (1967).

CHAPTER III

RESULTS AND DISCUSSION

The spermatogonial tissue of eight Chinese hamsters was examined. Table 1 divides the cells counted into the different chromosome counts and number of cells observed of each type in each hamster. The distribution of these data was expected to show some negative skewness because the squash method of slide preparation inherently causes cells to lose chromosomes. The skewness is demonstrated by the graph in Figure 2 of the compiled information from Table 1. Skewness can be measured by the formula $Sk = \frac{\bar{X} - Mode}{s}$, where $\bar{X}$ is the arithmetic mean and s is the standard deviation (Croxton, 1953). The $\bar{X}$ for the data in Table 1 is 21.49, s equals 0.77 and the Mode is 22. Consequently, Sk equals -0.66. The high value of Sk indicates the distribution is skewed and the minus sign indicates skewness to the left. The graph in Figure 2 was compared to a "normal" or Gaussian distribution by a Chi-square test which showed that the graph gives a very poor fit to the "normal." Ford (1964) noticed his data had pronounced negative skewness and attributed the phenomenon to a high incidence of broken cells which lost chromosomes. He also used a squash preparation technique. The assumption that cell breakage is the cause of the skewness may be valid because it is the only factor which could show such a large effect on the data. Aneuploidy could not possibly be a cause and in fact, as discussed earlier, this negative skewness emphasizes the point that only hyperdiploid cells are valid measurements of aneuploidy.

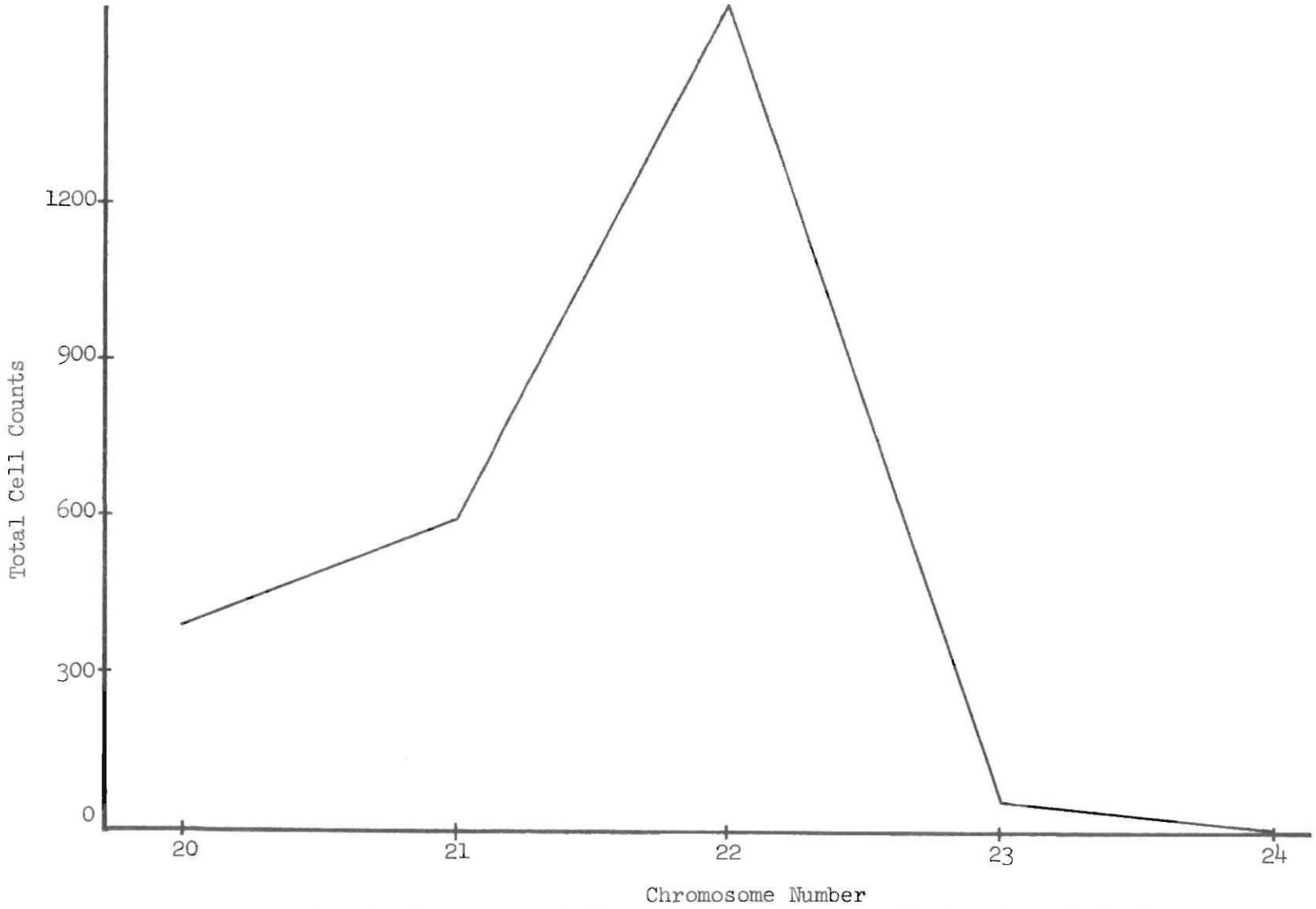

Figure 2. Graph of Total Cell Counts per Chromosome Number from Table 1.

A measurement of the "goodness of fit" of the data in Table 1 using a Chi-square test where marginal totals determine the expected frequencies was employed to determine the internal consistency of the data; it being accepted that the distribution is not described by the "normal," or Gaussian distribution. Table 2 shows the Chi-square values for each hamster as it relates to the rest of the data and a total Chi-square of 55.52. The probability of obtaining a value that high (where n, degrees of freedom, is 21) is less than 0.001. A probability of 0.05 or larger is considered an indication of a satisfactory fit (Croxton, 1953). Consequently, the data exhibits a very unsatisfactory internal consistency. However, if the table is studied closely, it is obvious that hamsters I and II contribute the most weight to the total Chi-square of 55.52.

To determine if hamsters I and II did fit with the rest of the data, the data were regrouped, first comparing hamster I and then hamster II with the remaining data using Chi-square tests with marginal totals as before. The Chi-square for hamster I versus all the other hamsters (where n is 3) was 9.1 and the Chi-square for hamster II versus all the others was 24.8. Using the same criterion as before (where p is 0.05) the Chi-square values must be less than 7.8 for a good fit. Therefore, hamsters I and II do not fit with the other six hamsters.

Table 3 is similar to Table 2 but hamsters I and II have been removed and another Chi-square performed on the remaining data. The total Chi-square (where n is 15) is 20.75 and the probability of obtaining that value is greater than 0.05 so now the observed and expected frequencies do fit together. The discrepancy of fit between Tables 2 and 3

Table 2. Chi-square Test of "Goodness of fit" using Marginal Totals from Data of Chromosome Counts of Normal Spermatogonial Tissue from Eight Chinese Hamsters.

Chromosome No.: / Hamster No.	Cell Count 20 f	20 fc	21 f	21 fc	22 f	22 fc	23 f	23 fc	$\Sigma \frac{(f-fc)^2}{fc}$
I	47	42.0	67	63.2	155	169.4	11	5.3	8.05
II	28	13.4	23	20.1	35	53.9	3	1.7	24.06
III	102	104.3	135	156.8	446	420.6	12	13.3	4.74
IV	64	73.1	119	109.9	296	294.7	8	9.3	2.07
V	17	12.8	19	19.2	46	31.4	3	1.6	3.17
VI	16	21.2	27	31.8	96	85.3	2	2.7	3.50
VII	73	70.1	125	106.5	268	285.6	6	9.0	5.40
VIII	46	55.5	76	83.5	243	223.9	5	7.1	4.53
Total	393	392.4	591	591.0	1585	1584.8	50	50.0	55.52

Table 3. Chi-square Test of "Goodness of fit" Using Marginal Totals from Data of Chromosome Counts of Normal Spermatogonial Tissue from Six Chinese Hamsters.

Chromosome No.:	Cell Count 20		21		22		23		$\Sigma \frac{(f-fc)^2}{fc}$
Hamster No.	f	fc	f	fc	f	fc	f	fc	
III	102	98.2	135	134.8	446	430.9	12	11.1	3.28
IV	64	68.8	119	108.4	296	301.9	8	7.8	1.49
V	17	12.0	19	18.9	46	52.7	3	1.4	4.74
VI	16	19.9	27	31.4	96	87.4	2	2.3	3.27
VII	73	66.7	125	105.1	268	292.6	6	7.6	6.77
VIII	46	52.3	76	82.4	243	229.4	5	5.9	2.20
Total	318	317.9	501	501.0	1395	1394.9	36	36.1	20.75

implies that my technique of slide preparation and chromosome counting improved with experience, hamsters I and II being the first hamsters studied cytologically.

Referring again to Table 1, a total of 2,619 cells from eight hamsters was observed. An extra large "marker" chromosome was found initially in 16 cells which were subsequently karyotyped for confirmation. Nine of these cells were found to have one extra "marker" chromosome of pairs one and two. Thus, the frequency of extra "marker" chromosomes was 1 in 291 cells. Since four chromosomes were involved, the frequency of trisomic aneuploidy for any one of the four "marker" chromosomes would be four times 291 or 1 in 1,164 cells. This number divided by 22 gives the frequency of trisomic aneuploidy produced by non-disjunction involving any one of the chromosomes of the Chinese hamster which is 1 in 53 cells. This frequency was calculated from the data in Table 1 before hamsters I and II were deleted. Using only the data from hamsters III through VIII, a frequency of 1 in 58 is obtained which is not much different from 1 in 53.

Based on the frequency 1 in 53 and the total number of cells examined, 2,619 divided by 53 gives 49 cells which should have been observed exhibiting a gain (trisomy) of any one of the 22 chromosomes. Table 1 shows that, of the 2,619 cells counted, 50 trisomic cells were observed involving any of the chromosomes. Thus, the total number of observed trisomics is approximately the same as the expected number based on the frequency of aneuploidy of 1 in 53. Moreover, when the frequency of 1 in 58 is used, the observed number of trisomics is 36 because hamsters I and II have been deleted and the expected number of trisomics

is 39. The agreement between these values using two different frequencies for aneuploidy implies that the large "marker" chromosomes can be counted with reliable accuracy and that total cell chromosome counts are reliable even if the majority of individual chromosomes are indistinguishable.

Ford (1964) maintains that normal somatic tissue demonstrates "remarkable constancy" with respect to chromosomal aberrations such as aneuploidy, while reports on Mongolism and this report on spermatogonial tissue show aneuploidy occurs more frequently. Ford bases his statement on the frequency of non-disjunction of the T6 chromosome in the mouse. He determined the minimal frequency of non-disjunction to be 1 in 17,500 for the T6 chromosome pair. Since there were two chromosomes at risk and a total of 40 chromosomes in the mouse karyotype, this represents a frequency of 1 cell in 875 for each chromosome to exhibit this type of aneuploidy (Ford, 1964). Nevertheless, Ford (1964) has earlier data from mouse somatic tissue showing a frequency of hyperdiploidy of 3 in 2,300 cells. This is a frequency of 1 in 767 cells or 1 in 38 cells if all 40 chromosomes in the mouse karyotype are considered. These figures agree fairly well with the results of this report as seen in Table 4 where the compiled data of this study is summarized and compared with the data of other investigators.

Mongolism, as was mentioned earlier, has been extensively studied and its frequency of occurrence can be correlated with the frequency of aneuploidy found in this study. Carter and MacCarthy (1951) in London hospitals found the frequency of Mongolism to be 1.51 per 1,000 births. Collman and Stoller (1962) found a frequency of 1.45 in Victoria, Australia, and Stark and Mantel (1966) found a frequency of 0.89 per 1,000

Table 4. Summary of Available Data on Aneuploidy

Cell System	Diploid Chromosome Number	Frequency of Aneuploids per Cell (Adjusted)*	Investigator
Mouse	40	1 in 38 1 in 875	Ford (1964)
Human	46	1 in 29	Carter and MacCarthy (1951)
		1 in 30	Collman and Stoller (1962)
		1 in 49	Stark and Mantel (1966)
Chinese Hamster	22	1 in 58	This study

*The frequencies of aneuploidy in the table are based on the total number of cells observed, the total number of aneuploid cells observed and the diploid number of chromosomes of the animal studied. These factors were different for each investigator.

live births in Michigan. Carter and MacCarthy (1951) point out that the actual frequency may be even higher than their calculations since nurses and physicians may not recognize Mongoloids when they are infants.

The frequencies of Mongolism that these investigators determined are averages of the occurrence of Mongoloid births from mothers of all ages. All of the investigators reported that the incidence of Mongolism increases with the age of the mother especially for mothers over 30. Consequently, a conservative frequency of 1 per 1,000 was chosen to compare the incidence of Mongolism with the incidence of aneuploidy in hamsters since young animals were used. A frequency of 1 per 1,000 for Mongolism is also the frequency of aneuploidy for chromosome number 21 in human beings as demonstrated by the work of Matsunaga (1966). Since human beings have 23 pairs of chromosomes, the frequency of 1 in 43 represents the occurrence of aneuploidy based on the incidence of Mongolism in human beings if all the chromosomes are considered. This frequency, Ford's earlier data (1 in 38) and this study (1 in 58) are in reasonable agreement. These data are graphed in Figure 3 which shows a negative correlation between the frequency of aneuploids per cell and the diploid chromosome number of the animals studied. The work of Blakeslee (1921 and 1922) and Darlington (1929) indicated that any chromosome of a cell could demonstrate non-disjunction to produce aneuploid daughter cells. Therefore, a cell with a large number of chromosomes would be expected to show a higher frequency of aneuploidy per cell than a cell with a small number of chromosomes. The agreement in the number observed and expected trisomics in this study, as discussed earlier, also supports this conclusion. Nevertheless, the graph in Figure 3 implies the opposite,

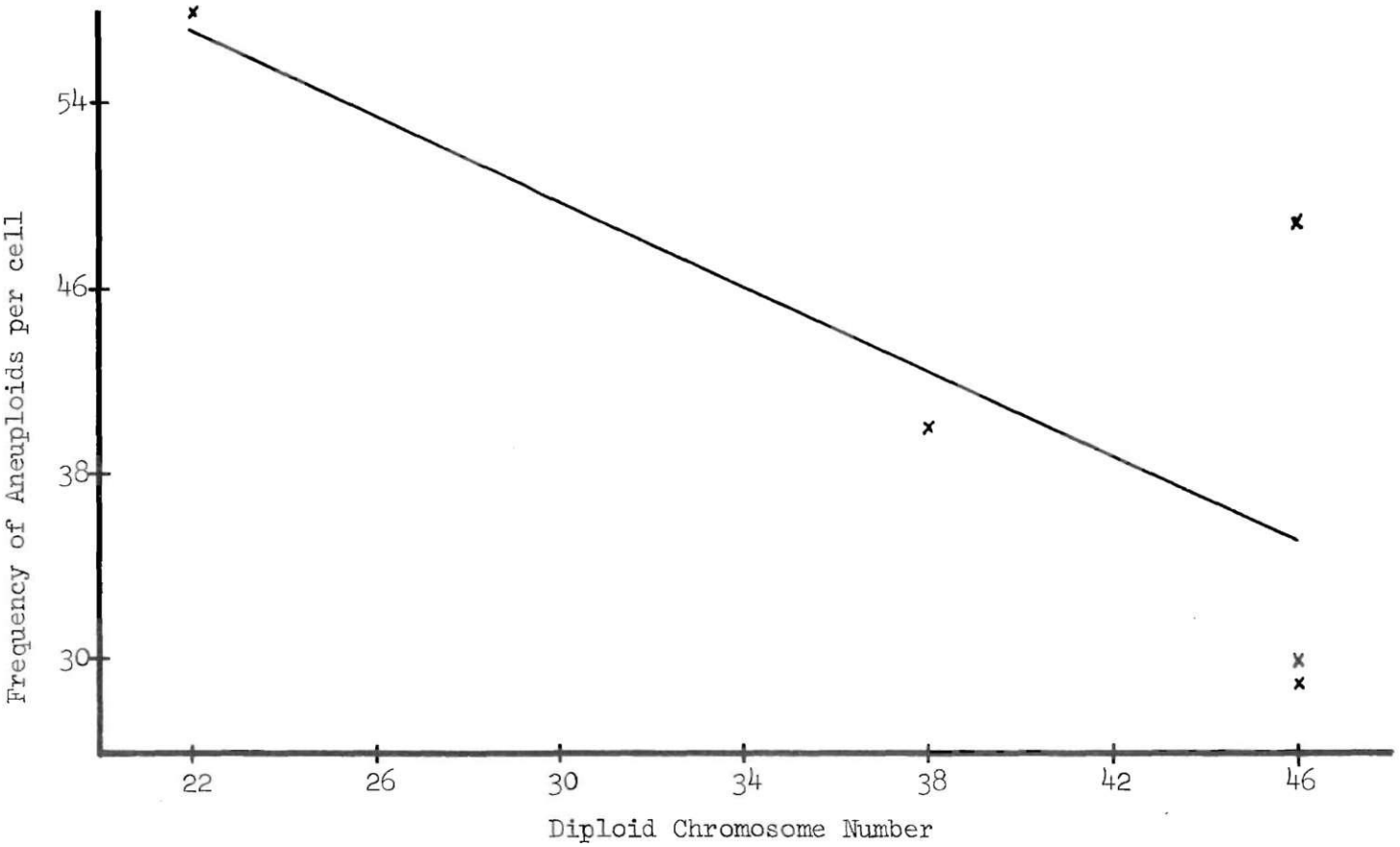

Figure 3. Graph of the Frequency of Aneuploids per cell versus the Diploid Chromosome Number of the Animals studied from Table 2.*

*Only the early data (1 in 38) of Ford (1964) was used.

that the frequency of aneuploidy decreases as the chromosome number of animals increases.

CHAPTER IV

CONCLUSIONS

A study of premeiotic preparations of spermatogonial tissue of Chinese hamsters and its correlation with data of other investigators led to the observations that:

1. The trisomic type of aneuploidy produced by the non-disjunction of chromosomes appears to occur approximately once in every 40 to 50 cells;
2. The concept that each of the individual chromosomes, regardless of size, has an equal probability of becoming aneuploid is supported by aneuploidy in the Chinese hamster.

CHAPTER V

RECOMMENDATIONS

The studies of Mongolism that were cited all agree that the incidence of aneuploidy resulting in Mongolism increases with the age of the mother. The hamsters used in this project were young (three to five months old) and it would be interesting to see if aneuploidy also increased with the age of hamsters, and whether aneuploidy occurs in other tissues, i.e., bone marrow. This is a very interesting subject and I recommend that more work be done in this area.

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